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Modulation of α4β1 and α5β1 Integrin Expression: Heterogeneous Effects of Q-Switched Ruby, Nd:YAG, and Alexandrite Lasers on Melanoma Cells In Vitro

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Background and Objective: Integrins of the β1 family are cellular adhesion molecules that play an important role in cell attachment and migration by interacting with extracellular matrix molecules. Agents such as hormones, cytokines, and ultraviolet radiation have all been shown to have an integrin modulating potential. The present study indicates that radiation of Q-switched lasers is also able to induce transient changes in integrin expression levels on human melanoma cells in vitro.
Study Design/Materials and Methods: Radiation from Q-switched Ruby (694 nm), Alexandrite (755 nm), and Nd:YAG laser (1,064 nm) with fluences comparable to those that are generally used in treating dermatologic lesions were used to irradiate a subconfluent layer of human melanoma cells. After fixed time intervals, the cells were harvested either to analyse the integrin expression by flow cytometry or to investigate changes in cell attachment, spreading, and migration.
Results: It was established that all three types of laser were able to cause a significant downregulation of both the α4 and the common β1 integrin subunit. The Alexandrite and Ruby lasers also induced a decrease in α5 expression; however, the cells treated with the Nd:YAG laser showed a marked upregulation of the α5 subunit. The expression of the other β1 integrin subunits was shown to be unaltered after laser treatment. Downregulation of the α4 upregulation of the α5 integrin subunit expression resulted in, respectively, decreased and increased attachment and spreading on fibronectin, the extracellular matrix ligand for both the α4β1 and α5β1 integrins. Marked upregulation of the α5 subunit also resulted in a higher migration rate.
Conclusion: Taken together, these results show that nonlethal doses of Q-switched laser radiation are able to induce changes in cellular behavior in vitro by modulating the integrin expression pattern.

Key words: laser, integrin, melanoma, migration, attachment, fibronectin

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INTRODUCTION

Integrins are ubiquitous cell surface proteins that mediate cell adhesion to extracellular matrix proteins as well as cell–cell interactions and are known to play a role in cell attachment, spreading, and migration. They consist of two subunits, one α and one β. Presently, at least 20 different combinations are known of the 14 α and 8 β chains discovered thus far. Each heterodimer binds to one or more ligands, including the extracellular matrix components fibronectin, laminin, and collagen. Integrins also have been shown to play an important role in the multistep process of invasion and metastasis of tumor cells [1, 2]. The structure and properties of this family of heterodimeric transmembrane adhesion molecules have been described extensively [3, 4]. The largest and most diverse group of integrins is the subfamily that shares a common β1 subunit, the so-called β1-integrins, which has been especially associated with cell attachment, spreading, and migration of normal and malignant cells, including melanoma cells [5–9].

Because of their important role in (tumor) cell migration, a considerable amount of studies has been performed on the mechanisms of expression and activation of integrins [4]. Among other agents, such electromagnetic energy sources as ultraviolet and gamma radiation have been implicated as potential integrin expression modulators [10, 11]. The possible modulating effects of laser radiation on integrin expression, however, have not been investigated thusfar. Given the fact that lasers are widely used in the treatment of dermatologic and other lesions and the important role integrins play in different aspects of cellular behavior, we deemed it necessary to investigate whether laser radiation is able to inflict changes in integrin expression. It was our intention to answer the following three questions: (1) does laser radiation influence integrin expression levels in cultured cells? (2) If so, is this modulation wavelength dependent? (3) Do these shifts in integrin expression induce effects on the in vitro cell attachment, spreading, and migration?

In order to answer these questions, we developed an assay in which cells of a well-characterized human melanoma cell line were exposed to different types of laser radiation. Flow cytometric analysis was used for detection of integrin expression and cell attachment assays as well as cell spreading, and migration assays were used to study possible changes in integrin-dependent cellular behavior.

MATERIALS AND METHODS

Melanoma Cell Culture

Lymph node-derived human metastatic melanoma cells (coded MM-RU) were maintained in culture as previously described [5, 6] In brief, cells were cultured in minimal essential media (MEM) with Eagle's balanced salt solution, containing 2% fetal calf serum (FCS) and 8% newborn calf serum (NCS), 75 units/ml penicillin, 50 mg/ml streptomycin, and amphotericin B (Gibco Cat No 6000-5240AG Antibiotic-Antimycotic). The cultures were passed at subconfluence approximately twice a week while maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Lasers and Laser Treatment

The lasers used in this study were a Q-switched Neodymium:YAG laser (Nd:YAG) at 1,064 nm (Continuum Biomedical Medlite, Sunnyvale, CA) with a peak power of 8 J/cm^2 and a pulse duration of 10 ns, a Q-switched Ruby laser (Ruby) at 694 nm (Spectrum Medical Technologies, Natick, MA) with a peak power of 10 J/cm^2 and a pulse duration of 20–40 ns, and a Q-switched Alexandrite laser (Alexandrite) at 755 nm (Candela Corp., Wayland, MA) with a 8 J/cm^2 peak power and a pulse duration of 100 ns.

In order to assess the integrin expression and functional behavior of the untreated cells and the cells treated with the different types of laser, cells were obtained in single cell suspension and at the same time plated in T-25 tissue culture flasks (Corning, New York, NY, cat #25106). At 24, 48, and 72 hours after plating the cells, the subconfluent cell monolayers were exposed to the different types of laser light.

Immediately prior to laser treatment, the tissue culture flasks were placed upside down, and laserlight was directed through the cell-covered plastic with the cell layer in the focal plane. Light blue paper, which changes color when hit by a laser beam, was placed under the flask to control for an even exposure of the entire cell layer. The lasers were used at the following parameters: the Nd:YAG laser with a 3.0 mm spot size and a fluence of 5.0 J/cm^2, the Ruby laser with a 5.0 mm spot size and a fluence of 5.0 J/cm^2, and the Alexandrite laser with a 3.0 mm spot size and 5.0 J/cm^2. The fluences were established using the readout on the lasers, as well as verified using an energy meter (Scientech, Bolder, CO). The rationale for choosing these parameters was

the fact that these energies are used clinically to irradiate pigmented lesions. In addition, we performed a series of experiments in which we used the Nd:YAG laser with fluences of 2, 4, 6, and 8 J/cm^2 with a 3 mm spotsize in order to study a possible dose-dependency. After laser exposure, the medium was removed, the cell layer was rinsed with phosphate-buffered saline (PBS) (37°C), and replaced with new medium. Dead cells present in the replaced medium were counted using a hemocytometer following trypan-blue staining. Treated and untreated cells were all harvested 96 hours after the initial cell plating.

Antibodies

The monoclonal antibody to the α5 integrin subunit (clone BIIG2) was kindly provided by Dr. Caroline Damsky (University of California, San Francisco). The monoclonal antibodies (mAbs) to the α2 (clone P1E6), α3 (P1B5), α4 (P4G9), and β1 integrin subunit (P4C10) were purchased from Gibco BRL (Gaithersburg, MD). The mAb to α6 (MAB1972) was from Chemicon (Temecula, CA). Fluoresceinated goat antimouse Ab (FL-GAM) (Cappel Laboratories, Durham, NC; cat. 55493-12110081) was used as secondary antibody to all mAbs, except to the α6 mAb, which required a fluoresceinated goat antirat Ab (FL-GAR, Rockland, Gilbertsville, PA; cat. 311-1202).

Flow Cytometry Analysis

At 24 hours after irradiation (for the time course study also 48 hours and 72 hours), both the laser-treated and untreated melanoma cell cultures were obtained in suspension after EDTA treatment of the subconfluent cultures, washed with PBS, and incubated for 30 minutes at 4°C with the mAbs described above at dilutions of 1:500 for all mAbs (from ascites) except for the anti-α5 mAb (from hybridoma supernatant) that was used in a 1:5 dilution. After washing three times with cold (4°C) PBS, the cells were incubated for 30 minutes with 1:40 FL-GAM (or FL-GAR for α6). After washing three times with cold PBS, the cells were fixed with 1% paraformaldehyde in PBS for 10 minutes and washed once with PBS. Flow cytometry analysis was performed by using a Becton Dickinson FACScan II. FL-GAM IgG alone was used as negative control.

Shifts in integrins after treatment with the laser were compared with the nonlaser-treated control cells and statistically analyzed by applying the Kolmogorov-Smirnov two-sample statistical analysis of histograms [12]. In each experimental condition and each individual integrin subunit tested, ~10,000 counts were obtained. In order to reject the null hypothesis that two cell populations are the same at the 99.5% confidence level ($\alpha = 0.001$), calculated D values must be larger than the D(n) critical value ($D_{crit} = 0.03$ for n = 10,000) events [12]. The greater the calculated D value (Dv) compared to the D_{crit}, the greater the shifts between the populations shown by the histograms will be apparent. In this study, we have chosen a range of arbitrary D values in order to categorize the detectable shifts in the histograms as follows: Absent or minimal shift if D values < 0.15; slight upward shift or slight downward shift; 0.15 < D value < 0.30; marked upward shift or marked downward shift: D value > 0.30. The D values were established by using LYSIS II version 1.1 statistical software (Becton Dickinson).

Attachment Assay

Fibronectin (FN; cat. 40008) was purchased from Collaborative Research (Bedford, MA). The bottoms of Lab-Tek 8 chamber slides (Nunc, Naperville, IL) were coated with FN at 10 μg/ml for 1 hour at 37°C following established techniques of glass substrate coating with extracellular matrix proteins [7]. Following this coating, the chamber slides were overlaid with 20 mg/ml heat-denatured (80°C for 20 minutes) bovine serum albumin (BSA) (Sigma, St. Louis, MO) for at least 12 hours at 4°C to block nonspecific binding sites on the microcover glass and matrix proteins. In each experiment, one of the chambers was coated with denatured bovine serum albumin (dBSA) alone in order to control for the specificity of the binding to FN.

Approximately 2.5×10^4 of either the laser-treated cells or the control cells in suspension were added per chamber in serum-free medium. After 30 minutes, the medium was removed, the plastic upper structure and the silicon gasket were detached from the slides, the coatings with the attached cells were vigorously washed with PBS and fixed with 10% formalin, stained with Gill's hematoxylin no.3 (Gibco BRL), and mounted with a coverslip. The number of attached cells was analyzed using the Microcomp Image Analysis system described below. The mean number of cells per 10^4 mm^2 was calculated by superimposition of a 100 mm grid on the video screen.

Cell Spreading Assay

Microcover glasses were coated with FN at 10 μg/ml for 1 hour at 37°C following the techniques described above. Cells were obtained in single cell suspension by treatment with EDTA 0.02% solution (Sigma) and plated on the fibronectin covered microcover glasses in serum-free medium. The cells were observed for a 2-hour period under a Nikon Diaphot inverted microscope with a 40× phase contrast objective in a hermetically sealed Plexiglass Nikon NP-2 incubator at 37°C in a 5% CO_2 and 95% air environment. At fixed intervals, the surface area of the cells were analyzed with the Microcomp image analysis system (Southern Micro Instruments, Atlanta, GA), a Numonic digitablet, a high resolution video monitor (Sony, New York, NY), and an IBM compatible pc (Samsung S500) equiped with a video card (PC vision plus frame grabber, Imaging Technology, Woburn, MA). The data were saved as an MS-DOS (Microsoft) file and translated through a network (TOPS, Sun Microsystem Co., Berkeley, CA) to an Apple Macintosh SE computer for statistical analysis (Students *t*-test, unpaired samples).

Cell Migration Assay

The melanoma cells were obtained in single cell suspension and seeded on fibronectin coated coverslips as described above. After 3 hours of incubation, the cells were placed in the Plexiglass incubator housing as described above, over an inverted microscope with a 10× phase contrast objective. Cell migration was recorded with a video camera attached to the video camera port and connected to a time-lapse video cassette recorder. In each experiment, the migration of at least 25 cells was observed for 4 hours. Video images were played back at 1-hour intervals and the digitally saved images were analyzed using a digitablet, a pc, and statistical software as described above.

RESULTS

Nd:YAG Exposure Causes Higher α5 and Lower α4 Integrin Subunit Expression

The exposure of the melanoma cells to laser radiation resulted in only scant immediate cell death in every experiment (<0.1%). Cell mortality between the time of irradiation and the moment of harvesting for analysis was not significantly different from the control cells for this period. In all the other conditions, no significant increase in cell mortality could be detected.

At 24 hours after treatment with the different types of lasers, the cells were processed and analyzed using flow cytometry as described in Materials and Methods. No significant changes in integrin expression could be detected in the α1, α2, α3, and α6 subunits following treatment with any of the three lasers. In contrast, cells treated with the Nd:YAG laser showed a significantly higher α5 integrin subunit expression (MFI (mean fluorescence intensity) = 17.5; Dvalue = 0.30) as compared to the control (MFI = 13.0) (see Fig. 1, Table 1). The shift in α5 expression at 48 hours is less significant (MFI = 16.9; Dv = 0.26), but the difference is marked at 72 hours (MFI = 24.0; Dv = 0.57). Except for the 48-hour time point, these α5 integrin shifts are not accompanied by significant changes in the β1 subunit expression. Eight days after exposure to the Nd:YAG laser, the shift in α5 integrin is no longer significant (MFI = 15.4; Dv = 0.12). The cells treated with the Ruby laser exhibited only slight downward shifts of the α5 integrin subunit at 24 and 72 hours after irradiation. Cells irradiated with the Alexandrite lasers showed no significant changes in α5 integrin expression. The increase in α5 integrin subunit expression 72 hours after Nd:YAG irradiation appeared to be a dose-dependent phenomenon (Fig. 2; see also Fig. 5).

Cell treatment with the Ruby and Alexandrite lasers resulted in a marked downregulation of the α4 integrin subunit after 24 hours (MFI = 13.1; Dv = 0.40 and MFI = 13.2; Dv = 0.43, respectively, control MFI = 21.0) and 48 hours (MFI = 13.5; Dv = 0.37 and MFI = 13.3; Dv = 0.42). After 72 hours, the α4 levels were recovering in both the Ruby laser (MFI = 17.6; Dv = 0.16) and Alexandrite laser (MFI = 17.1; Dv = 0.19) treated cells. Cells treated with YAG laser showed a slight downward shift in α4 subunit expression (MFI = 15.1; Dv = 0.29). After 48 hours, however, the α4 expression decreased further to levels significantly lower than those of the the controls (MFI = 13.6; Dv = 0.41) but appeared to be recovering at 72 hours after YAG-treatment (MFI = 16.5; Dv = 0.24).

The β1 subunit expression at 24 hours after treatment showed shifts that resembled the shifts in the α4 subunit. A very significant decrease was noted in the cells treated with the Alexandrite and Ruby lasers (MFI = 57.7; Dv = 0.42 and MFI = 63.7; Dv = 0.30, respectively, control MFI = 85.3). At t = 48 and t = 72 hours, the shifts in β1

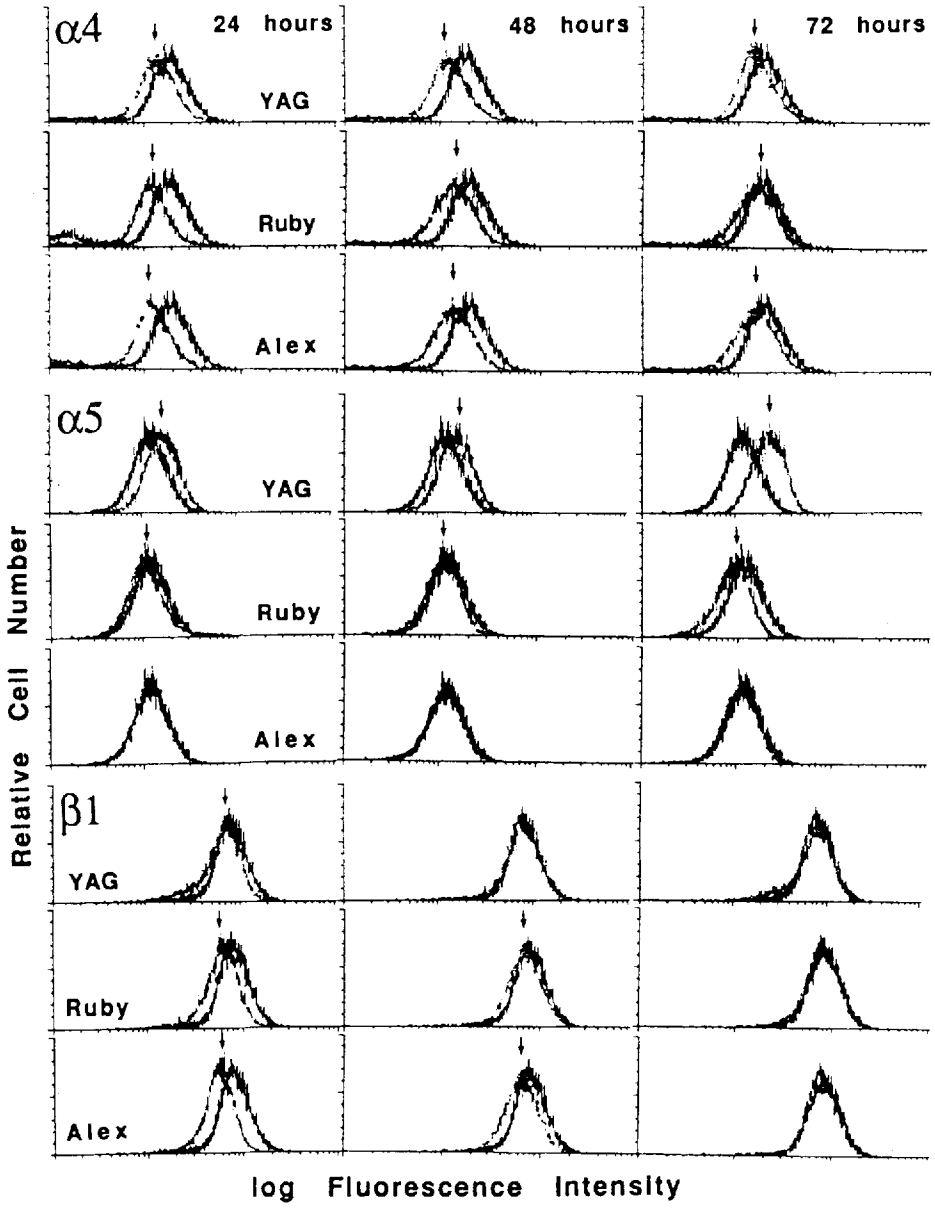

Fig. 1. Graphic representation of the α4, α5, and β1 integrin subunit expression upon laser treatment. The graphs drawn with bold lines represent the expression of the untreated control cells, whereas the graphs in regular lines show the expression after laser treatment. The peaks of the graphs representing the treated cells are marked with an arrow.

integrin subunit expression were slight or absent in cells treated by all the three lasers.

Cell Attachment, Spreading, and Migration on Fibronectin are Enhanced in YAG Laser-Treated Cells

After 40 minutes of incubation, the melanoma cells irradiated with the Alexandrite and Ruby lasers 24 hours previously showed a decrease in attachment on fibronectin (FN) as compared to the untreated controls ($P < 0.05$) (Fig. 3). However, cells harvested after 48 or 72 showed no significant alteration in attachment characteristics. Cells treated with the YAG laser attached significantly better to FN at 24 ($P < 0.05$) and 72 hours ($P < 0.001$) after irradiation as compared to the control (Fig. 3).

At t = 24 hours, the cell spreading assay showed a significantly lower projected cell surface area in cells irradiated with the Alexandrite la-

TABLE 1. Expression of Integrin Subunits on MMRU Cells Treated With Different Types of Laser: Mean Fluorescence Intensity Values (MFI)*

Laser		α2	α4	α5	β1
Control		24.9	21.0	13.0	85.3
YAG	24 hour	20.7	*15.1*	**17.5**	82.9
	48 hour		**13.6**	*16.9*	*68.5*
	72 hour		*16.5*	**24.0**	79.6
Ruby	24 hour	22.8	**13.1**	*12.1*	**63.7**
	48 hour		**13.5**	12.3	74.6
	72 hour		*17.6*	*11.3*	83.3
Alex	24 hour	23.2	**13.2**	12.5	**57.7**
	48 hour		**13.3**	12.7	*66.7*
	72 hour		*17.1*	12.5	84.0

*Bold MFI values indicate marked shifts in expression; italic MFI values indicate slight shifts (Kolmogorov-Smirnov two-sample test outlined in Materials and Methods). Shifts can be upward or downward; all italic and bold numbers >99.5% confidence level (α = 0.001).

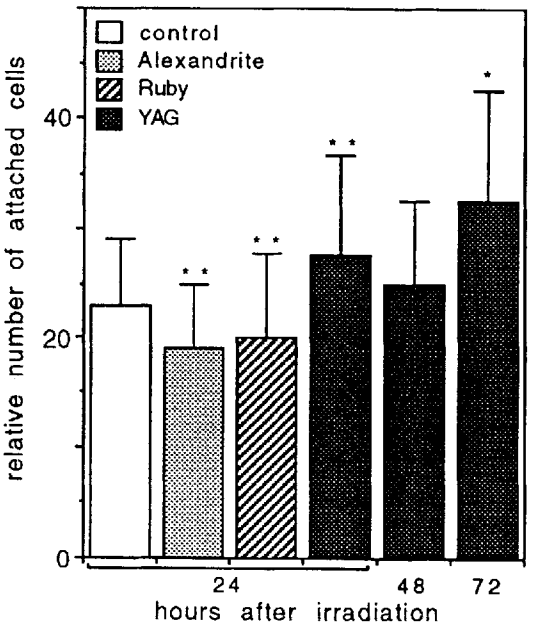

Fig. 2. Attachment of MMRU melanoma cells on a 10 mg/ml coating concentration of fibronectin. Attachment of cells treated with Alexandrite and Ruby lasers was significantly lower ($P < 0.05$) and with YAG laser significantly higher ($P < 0.05$) after 24 hours as compared to the untreated controls. At 72 hours after YAG-treatment, the increase in attachment was highly significant, as compared to the control ($P < 0.001$). Y-error bars represent standard deviation.

sers as compared to the control (Fig. 4). A significant increase in projected cell surface area was noted in the YAG treated cells at 72 hours ($P < 0.001$) after irradiation, with a mean projected cell surface area rising from 390 to 500 μm^2. The

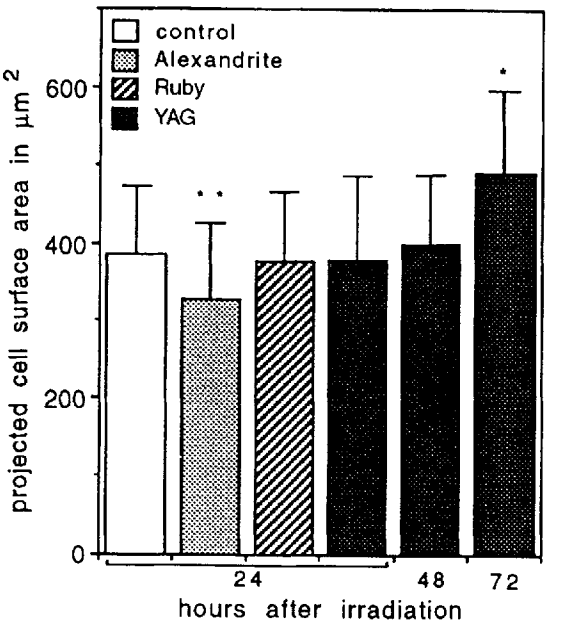

Fig. 3. Spreading of MMRU melanoma cells on a 10 mg/ml coating concentration of fibronectin. Mean projected cell surface area was significantly decreased in cells that were exposed to the Alexandrite laser 24 hours previously ($P < 0.05$). No change projected cell surface area could be detected 24 hours after treatment with Ruby and YAG lasers as compared to untreated control cells. However, 72 hours after YAG laser exposure a significant increase in projected cell surface area occurred ($P < 0.001$). Y-error bars represent standard deviation.

Ruby laser induced no significant change in cell surface at any time (Fig. 4).

Migration studies performed on the cells treated with Alexandrite and Ruby lasers after 24, 48, and 72 hours showed no significant change in migration rates on FN (Fig. 5). Likewise, no significant increase in mean migration rate could be observed in the cells 24 and 48 hours after exposure to Nd:YAG laser radiation. After 72 hours, however, the increase in migration rate of Nd:YAG laser-treated cells on fibronectin was significantly higher as compared to the control cells ($P < 0.001$).

DISCUSSION

The ability of cells to attach, spread, and migrate depends on the interaction of extracellular matrix proteins such as fibronectin, collagen, and laminin, and the repertoire of integrin adhesion molecules on the cell surface. The β1-integrins are considered to be prominent members of the integrin adhesion molecule family that play an

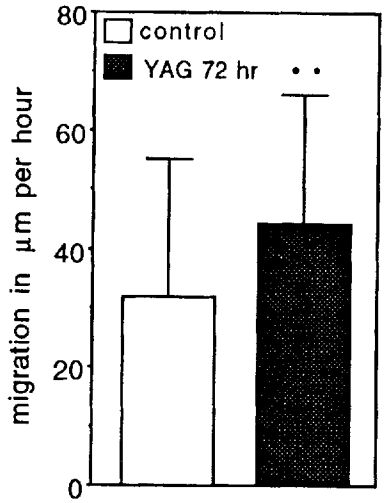

Fig. 4. Migration of MMRU melanoma cells on a fibronectin-coated coverslip (10 mg/ml coating concentration) in micrometers per hour. The migration was significantly increased at t = 72 hours after YAG treatment ($P < 0.05$). Migration rates were not significantly altered by exposure to the Alexandrite and Ruby lasers, or with the YAG laser after 24 and 48 hours. Y-error bars represent standard deviation.

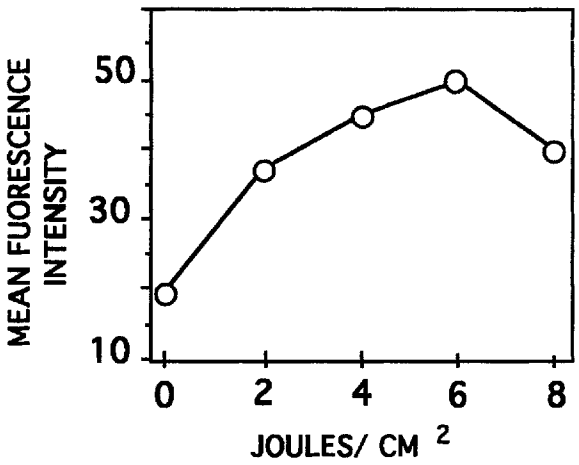

Fig. 5. The α5 integrin expression on the melanoma cells as measured with flow cytometry analysis, 72 hours after irradiation with the Nd:YAG laser at different fluences. A distinct dose-response relationship was observed.

important role in tumor cell invasion and metastasis [1]. Changes in either integrin expression or integrin activation state may induce functional changes in cell behavior, which, in the case of tumor cells, may have far-reaching consequences because of enhanced migration and invasion potential. Although the modulating properties toward adhesion molecule expression of a number of agents have been studied, no data exist whether laser radiation can induce integrin expression changes.

In our in vitro laser treatment assay, subconfluent melanoma cell layers were exposed to laser radiation, using fluences comparable to those used in treatment of pigmented lesions in patients [13]. In this assay, the immediate cell death inflicted by the radiation was low, despite the fact that the cells were located in the focal plane of the laser beam. One reason for the minimal cell death might be the fact that the cells of the human melanoma line used in the experiments contained little pigment, the major target and energy absorber of the wavelengths of the lasers that were used in this study.

The data on integrin expression show a diminished presence of the common β1 integrin subunit on the cell surface after 24 hours, which reflects a decrease in the total number of β1 integrins in cells treated with any of the three lasers. In contrast to a general decrease in α4 subunit expression in all three lasers, a specific and highly significant upregulation of the α5 subunit was found in the Nd:YAG laser-treated cells. The Nd:YAG-laser was found to be capable of inducing a higher expression level of the α5 integrin subunit, whereas radiation of the Ruby laser resulted in a lower expression of the same subunit. The data suggest that integrin modulation by Q-switched lasers is partly specific. Differences in modulation properties can be due either to the light itself or to differences in heating of the cells following laser-dependent energy transfer. Heat and ultraviolet light in the UVB range cause a generalized suppression of cellular protein synthesis [14, 15]. If the laser irradiation indeed heats the cells temporarily, this may explain the significant downregulation of the β1 and α4 integrin subunits. It is also well documented that heat stress, ultraviolet light, and a number of toxic agents can induce the synthesis of the so-called heat shock proteins or stress proteins [16, 17]. It is possible that the laser irradiation causes a sudden and transient heating of the melanoma cells and that the subsequent upregulation of the α5 integrin subunit can be regarded as a heat-stress-related event. However, this would have to be a specific effect since the other integrin subunits do not shift accordingly. Furthermore, this cell heating, if it occurs at all, cannot be measured and is of only very short duration compared to heat exposure times of 15 minutes to 1 hour in regular stress protein expression experiments [18]. In addition, experiments by Polla and

Anderson [19] showed that CO_2 lasers can not induce heat-shock effects in cultured fibroblasts. One could hypothesize that in our assay laser treatment may select a subpopulation with a preexistent different integrin expression level. However, in view of the very limited number of cells killed after laser irradiation, this appears highly unlikely. Furthermore, the expression of integrins returns to pretreatment levels after 8 days.

The shifts in cellular integrin expression may be due either to conformational shifts in existing surface molecules or due to a synthesis de novo. The mechanism through which the α5 integrin subunit upregulation occurs is not yet known, and future experiments designed to address this question are under investigation. Our present study identified specific shifts in expression and we focused on whether these shifts related to functional differences in cell behavior.

A high expression of extracellular matrix (ECM) receptors has been found to correlate with increased cell attachment and migration on ECM-ligands that specifically bind to those receptors. The two β1-integrins that were shown to be modulated by the laser, the α4β1 and α5β1, are both major cell surface receptors for fibronectin. In the present study, the simultaneous decrease in α4β1 and α5β1 in cells treated with the Alexandrite and Ruby lasers resulted in a significant lower rate of attachment to fibronectin of cells irradiated with these two lasers. A decrease in mean projected cell surface area in the Alexandrite-treated cells was observed. In the Nd:YAG laser-treated cells, the downregulation of the α4β1 appears to have been functionally compensated by the increase in α5β1 expression, since cell spreading after 24 hours in both attachment and spreading after 48 hours showed no significant difference as compared to the controls, presumably because of this balance in fibronectin-receptor shifts. After 72 hours, however, the increase in α5 appears to dominate the decrease in α4, since this resulted in a significantly higher cell attachment to fibronectin as well as an increased mean projected cell surface area. Changes in α4 and α5 subunits were in most cases apparently not sufficient to bring about significant alterations in the cellular migration rates on fibronectin; however, 72 hours after Nd:YAG laser treatment with marked upregulation of the a5 subunit, a significant increase in migration was detected.

This study shows that laser irradiation, when applied in nonlethal doses to melanoma cells in vitro, is able to alter the expression pattern of β1 integrins and shows also that this modulation of expression is sufficient to influence cellular functions such as attachment, spreading, and migration. All three lasers appear to be capable of inducing lower integrin levels, whereas the Nd:YAG laser is also capable of inducing higher levels of integrin expression. At this point, it is unclear whether the observed effects are solely a wavelength dependent phenomenon, or whether, e.g., variations in pulse width may influence these effects. All integrin expression changes were transient and are back to pretreatment levels 8 days after radiation.

In order to establish the clinical significance of these findings, further investigations into the mechanisms behind the shifts in integrin expression, the effects of laser irradiation on other adhesion molecules and the modulating potential of lasers in cell types other than melanoma cells will be necessary. In vivo studies of changes in cellular integrin expression in response to laser treatment are currently in progress and may, together with the proposed in vitro studies, provide further insight in the cell function modulating capacities of clinically applied lasers.

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REFERENCES

1. Ruoslahti E, Giancotti FG. Integrin and tumor cell dissemination. Cancer Cells 1989;1:119–126.
2. Albelda SM. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. Lab Invest 1993;68:4–17.
3. Hemler ME. VLA proteins in the integrin family: Structures, functions, and their role on leukocytes. Annu Rev Immunol 1990;8:365–400.
4. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.
5. Byers HR, Etoh T, Doherty JR, Sober AJ, Mihm JMC. Cell migration and actin organization in primary, recurrent cutaneous, and metastatic melanoma cell lines. Time lapse and image analysis. Am J Path 1991;139: 423–435.
6. Etoh T, Thomas L, Pastel-Levy C, Colvin RB, Mihm MCJ, Byers HR. Role of integrin alpha-2 beta-1 (VLA-2) in the migration of human melanoma cells on laminin and type IV collagen. J Invest Dermatol 1993;100:640–647.
7. Yamada KM, Kennedy DW, Yamada SS, Gralnick H, Chen WT, Akiyama SK. Monoclonal antibody and syn-

thetic peptide inhibitors of human tumor cell migration. Cancer Res 1990;50:4485–4496.
8. Vink J, Thomas L, Etoh T, Bruijn JA, Mihm MC, Gattoni-Celli S, Byers HR. Role of Beta-1 integrins in organ specific adhesion of melanoma cells in vitro. Lab Invest 1993;68:192–203.
9. Hall DE, Reichardt LF, Crowley E, Holley E, Moezzi H, Sonnenberg A, Damsky CH. The alpha-1/beta-1 and alpha-6/beta-1 integrin heterodimers mediate cell attachment to distinct sites on laminin. J Cell Biol 1990;110: 2175–2184.
10. Onoda JM, Piechocki MP, Honn KV. Radiation-induced increase in expression of the alpha IIb beta 3 integrin in melanoma cells: effects on metastatic potential. Radiat Res 1992;130:281–288.
11. Tang A, Udey MC. Inhibition of epidermal Langerhans cell function by low dose ultraviolet B radiation. Ultraviolet B radiation selectively modulates ICAM-1 (CD54) expression by murine Langerhans cells. J Immunol 1991;146:3347–55.
12. Young IT. Proof without prejudice: Use of the Kolmogorov-Smirnov test for the analysis of histograms from flow systems and other sources. Histochem Cytochem 1977;25:935–941.
13. Anderson RR, Levins PC, Grevelink JM. Lasers in dermatology. In: Fitzpatrick TB, ed. "Dermatology in General Medicine." New York: McGraw-Hill, 1993, pp 1755–1766.
14. DiDomenico BJ, Bugaisky GE, Lindquist S. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Proc Natl Acad Sci USA 1982;79:6181–6185.
15. Epstein JH, Fukuyama K, Fye K. Effects of ultraviolet radiation on the mitotic cycle and DNA, RNA and protein synthesis in mammalian epidermis in vivo. Photochem Photobiol 1970;12:57–65.
16. Herrlich P, Angel P, Rahmsdorf HJ, Mallick U, Poting A, Hieber L, Lucke-Huhle C, Schorpp M. The mammalian genetic stress response. Adv Enzyme Regul 1986;25:485–504.
17. Lindquist S. The heat shock response. Annu Rev Biochem 1986;55:1151–1191.
18. Maytin EV. Differential effects of heat shock and UVB light upon stress protein expression in epidermal keratinocytes. J Biol Chem 1992;267:23189–23196.
19. Polla BS, Anderson RR. Thermal injury by laser pulses: Protection by heat shock despite failure to induce heat-shock response. Lasers Surg Med 1987;7:398–404.